Assessing Phytoplankton Composition, Abundance, and Biomass and HAB Relationships to Chlorophyll *a* of the James, Elizabeth and Lafayette Rivers: 2013 monitoring season.

Data Report
To Virginia Department of Environmental Quality
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By

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Introduction

Phytoplankton community composition and biomass have long been used as bio-indicators of water quality as they represent the effect of environmental conditions over an extended period of time on living resources. Due to the relative ease of measurement, concentrations of the pigment Chlorophyll *a* (Chl *a*) are often utilized as a proxy measure for algal biomass standing stock (Boyner et al. 2009). By associating Chl *a* concentrations with environmental conditions, and ecosystem impairments, numeric Chl *a* criteria can be established as indicators and management goals for water bodies, including Chesapeake Bay (Harding et al. 2013). However, a concern with this method is the considerable range in Chl *a* concentrations, cell size, and carbon content (biomass) between species, which can vary by orders of magnitude (Boyer et al. 2009). Variability in environmental conditions (temperature, nutrients, light, etc.) are associated with changes in phytoplankton composition, as competition favors certain taxa both seasonally and spatially. In Chesapeake Bay and its tributaries, long-term algal monitoring has identified a succession of dominant algal groups that are prevalent in different regions throughout the year (Lacouture et al. 2006, Marshall et al. 2006). Included within the seasonal succession of phytoplankton taxa, are the presence of harmful algal blooms (HABs), which generally are increased populations of individual species or groups that are associated with a detrimental symptom (i.e. low dissolved oxygen, toxicity, etc.) (Smayda 1997, Marshall et al. 2009, Marshall and Egerton 2009). As different species and taxonomic groups vary in their Chl

a and biomass as well as their life history (toxicity, growth rates, etc.), it is vital to understand how algal composition, both HABs and non-HABs, not just Chl *a*, responds to environmental variability. To develop a predictive understanding of HABs and their complex interaction with environmental conditions and the rest of the plankton community, species-specific data is required (Pitcher 2012).

To evaluate the relationships between Chl *a*, environmental conditions and phytoplankton community composition in the tidal James River, algal populations were monitored as a component of Virginia Department of Environmental Quality's (DEQ) review of the numeric Chl *a* criteria. Long-term routine monitoring of water quality parameters, Chl *a* concentrations and phytoplankton populations in Virginia tidal waters including James River by DEQ and its Chesapeake Bay Monitoring Program partners (Old Dominion University and others) has been carried out at fixed stations on a monthly interval since 1986 (Marshall & Alden 1990, Marshall et al. 2003, Dauer et al. 2012). These data have led to a better understanding of seasonal dynamics, long term changes, and characterization of the phytoplankton community within the James River and other Virginia tributaries (Marshall et al. 2005, Nesius et al. 2007, Marshall et al. 2009). However, due to the spatial patchiness and ephemeral nature intrinsic to HABs and phytoplankton in general, the magnitude, frequency and duration of blooms can be underrepresented or missed altogether by routine monitoring (Morse et al. 2011, Egerton et al. 2012). To better capture the spatial and temporal variability present in these dynamic ecosystems, a higher frequency monitoring strategy was employed in the tidal James River beginning in 2011.

Results of the 2011 and 2012 monitoring season indicated that Chl *a* was significantly positively correlated with algal biomass in both the upper and lower James River. However there were significant differences in species composition between the river segments with diatoms and chlorophytes being the major contributors of biomass in the tidal fresh segments, and dinoflagellates and diatoms in the meso/polyhaline segments. Major dinoflagellate blooms of *Heterocapsa triquetra* and *Cochlodinium polykrikoides* in the lower James River and its tributaries, including the Elizabeth and Lafayette Rivers were responsible for the highest biomass and Chl *a* concentrations observed. In contrast, the upper James was characterized by a more stable, diverse community of diatoms, green algae and other taxa. In addition to *C.*

polykrikoides, five other HAB species were recorded in the James River, including cyanobacteria in the upper James and dinoflagellates in the lower James. The *C. polykrikoides* bloom initiation appeared to occur in the Lafayette River, with tidal transport of the bloom into the surrounding estuaries. The blooms observed in 2012 were larger and more extensive than previous years and possibly associated with high levels of regional storm activity and elevated temperatures. Continued monitoring of the phytoplankton community, with a concentration on initiation factors, bloom magnitude and duration was recommended to study the effects of interannual variability in environmental conditions. This report documents the methods and results of the 2013 monitoring season.

Major objectives of this study

1. Identify the phytoplankton populations, including both HAB and non-HAB species that are present and contributing to the chlorophyll concentrations in the James, Elizabeth, and Lafayette rivers throughout the sampling period.
2. Determine specific chlorophyll relationships to the biomass, composition, and abundance of the phytoplankton populations in these rivers.
3. Identify seasonal chlorophyll linkages to algal bloom occurrences throughout the study, emphasizing both HAB and non-HAB algal bloom producers.
4. Evaluate HAB species composition in these waters relative to their presence and concentrations that would exceed recognized threshold levels implying potential harmful/toxic conditions.
5. Describe the temporal and spatial extent of occurring blooms, emphasizing dominant taxa and functional algal groups, including their concentrations, biomass, duration, and frequency in these rivers, and changes over these and other parameters.
6. Compare and evaluate results obtained to recent and historical data to provide a more comprehensive analysis of the phytoplankton/chlorophyll dynamics and relationships in these rivers.
7. Identify status and any specific concerns regarding (the HAB) *Cochlodinium polykrikoides* presence, or dynamics in the lower James River estuarine complex, as well as, any growing concerns regarding other HABs in these waters.

Methods

Surface water samples (500ml) were collected in the upper James River (Upper and Lower Tidal Fresh segments) by personnel from Virginia Commonwealth University, under the supervision of Dr. Paul Bukaveckas and by DEQ Peidmont Regional Office supervised by Mr. Louis Seivard, and forwarded to ODU for analysis. These were tidal freshwater sites, although subject to periods of low saline intrusion. Samples were collected at DEQ stations TF5.3 and TF5.5 weekly from May through October and monthly in August and September at stations TF5.2, TF5.2A, TF5.5A, and TF5.6 (Fig.1, Table 1). A total of 68 samples were collected and analyzed during 2013 from the upper James River. Chlorophyll measurements were taken by VCU and DEQ personnel following the methods and protocol described by Wood et al. (2013), with this data forwarded to ODU for correlations with the phytoplankton data.

Water samples from the lower James River were collected by personnel from the Hampton Roads Sanitation District (HRSD) under the supervision of Mr. Will Hunley. These were surface (<1m) samples (125ml) taken weekly from February through October, in the mesohaline and polyhaline James River, as well as the Elizabeth and Lafayette Rivers (Fig. 1, Table 1). This included weekly collections from 7 fixed DEQ stations (LE5.1, LE5.2, LE5.3, LE5.4, LE5.5-W, LE5.6, and LFB01) and additional collections based on in-situ DATAFLOW chlorophyll readings. The threshold value for bloom recognition and sample collection were chlorophyll readings >15 μgL^{-1} (Egerton et al. 2012). 350 samples collected by HRSD were analyzed in 2013. All samples were preserved immediately with Lugol's solution and delivered to the ODU Phytoplankton Analysis Laboratory for analysis (Marshall et al. 2005). Chlorophyll concentrations determined by HRSD were provided to determine chlorophyll relationships to phytoplankton composition, biomass, and abundance levels. Chl *a* measurements were made by HRSD staff using the DATAFLOW system, following the methods and protocol described by Moore et al. (2013). When requested, non-preserved water samples were also provided for analysis.

Daily collections of water samples were obtained from the Lafayette River by Dr. Katherine Filippino from the ODU Department of Ocean, Earth and Atmospheric Sciences to target bloom initiation factors. Analysis of 87 samples comprised of collections over a ~60 day period in June, July and August at two stations; one near the mouth of the Lafayette River at the Norfolk Yacht and Country Club (NYCC), and one upstream at Ashland Circle (AC) (Fig.2).

Phytoplankton composition and concentration determinations followed standard protocols using light microscopy as followed in the Chesapeake Bay Phytoplankton Monitoring Program (Marshall et al. 2005). The Old Dominion University phytoplankton analysis laboratory contains extensive identification references, with a staff represented by T. Egerton, H. Marshall, plus 4 graduated research assistants. A scanning electron microscope facility, molecular laboratory, and cell culture room are located nearby and available for use. The methods and QA/QC standards followed are indicated in the 2012 "Work Quality Assurance Project Plan for Monitoring Phytoplankton and Picoplankton in the Lower Chesapeake Bay and Tributaries" (Marshall 2012). Biomass estimates were based on species specific biovolumes (μm^3) and converted to carbon (µgC/L) according to Smayda (1978). Pearson correlation analysis was used to compare chlorophyll (µg/L) to algal biomass of total phytoplankton concentrations, major group totals, and major species.

Phytoplankton diversity was quantified as species richness (number of unique algal taxa per sample) and species evenness (Pielou's evenness index, J) (Pielou 1966, Filstrup et al. 2014). Evenness values range from 0 to 1 with higher values indicating higher evenness, as is calculated based on biomass values. Evenness here refers to the relative distribution of biomass between species. For example during a bloom in the lower James River, *Cochlodinium polykrikoides* can contribute >95% of the total algal biomass. The evenness of these communities during the bloom is very low (0.01). In comparison, during non-bloom periods in the same river segments, there is a more even distribution of biomass amongst a larger number of co-existing species in the phytoplankton community with evenness values of 0.8 and higher. Virginia DEQ and the US EPA have stated that favorable conditions are those that allow for a balanced assemblage of natural resources. Greater diversity has been associated with increased levels of ecoystem productivity and stability including resistance and resilience to disturbances (Korhonen et al. 2011, Egerton 2013,). Regarding phytoplankton, diverse communities are considered to be more stable and less likely to collapse than those that are dominated by a single bloom species (Smith 1985). Chesapeake Bay phytoplankton can be characterized as one of relatively high species richness and low evenness, with a large number of background species, and a relatively small number of dominant taxa (Egerton 2013).

Table 1: Fixed station coordinates.

River Segment	Station Name		Latitude (decimal deg.)	Longitude (decimal deg.)
	ID	River Mile		
James River Upper Tidal Fresh	TF5.2	JMS119.30	37.5305	-77.434
James River Upper Tidal Fresh	TF5.2A	JMS104.16	37.4500	-77.4188
James River Upper Tidal Fresh	TF5.3	JMS099.30	37.40348	-77.3926
James River Lower Tidal Fresh	TF5.5	JMS075.04	37.31245	-77.2339
James River Lower Tidal Fresh	TF5.5A	JMS69.08	37.30165	-77.1284
James River Lower Tidal Fresh	TF5.6	JMS055.94	37.27272	-76.9906
James River Oligohaline	RET5.2	JMS042.92	37.20294	-76.78219
James River Mesohaline	LE5.1	JMS032.59	37.20297	-76.64833
James River Mesohaline	LE5.2	JMS021.04	36.05600	-76.59306
James River Mesohaline	LE5.3	JMS013.10	36.99044	-76.47544
James River Polyhaline	LE5.4	JMS005.72	36.95486	-76.39275
James River Polyhaline	LE5.5-W	0	36.99903	-76.313278
Elizabeth River	LE5.6	ELI002.00	36.90456	-76.33836
Lafayette River	LFB01	LAF003.83	36.8894	-76.2814
Lafayette River	NYCC		36.9064	-76.3059
Lafayette River	AC		36.8803	-76.2724

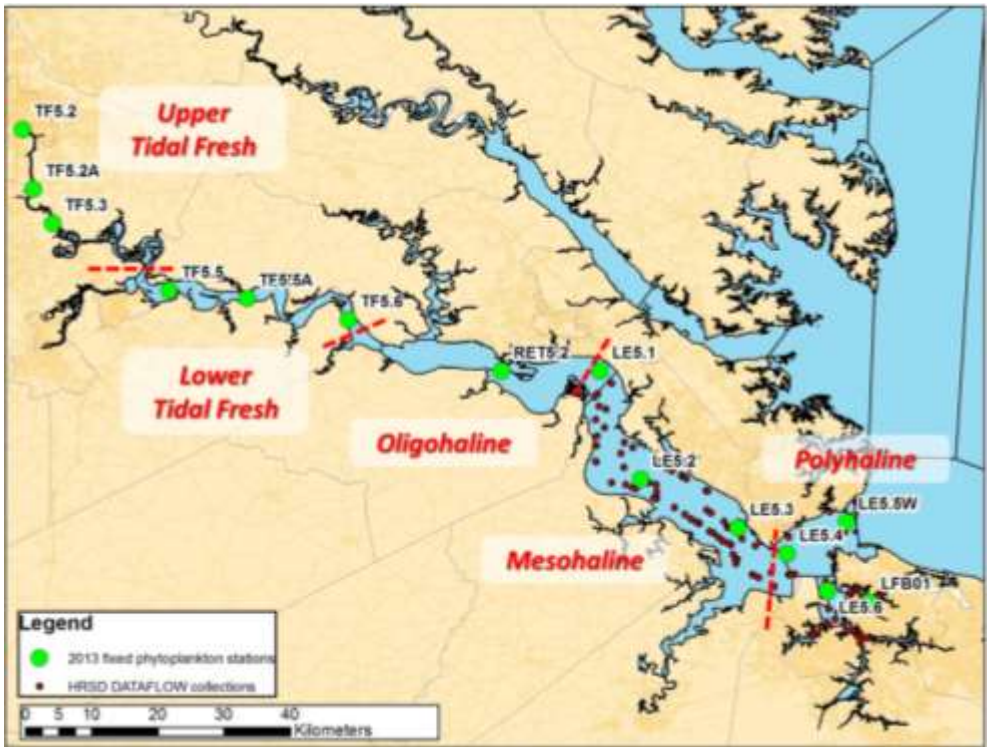

Figure 1: 2013 James River phytoplankton collections. Fixed sites shown in green. HRSD DATAFLOW bloom collections in red.

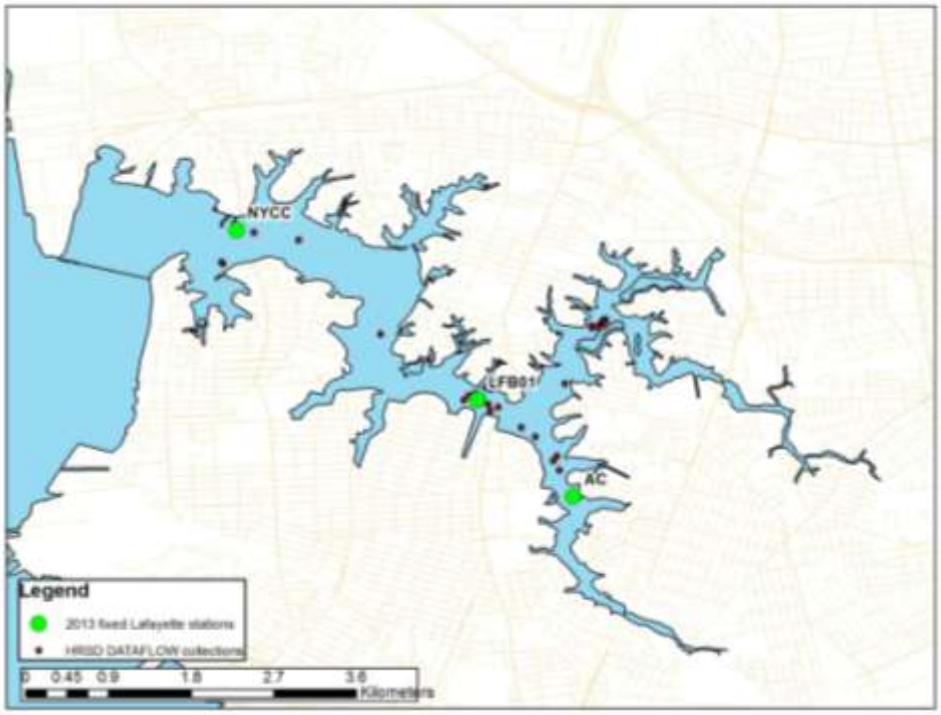

Figure 2: Lafayette River phytoplankton collections. Fixed sites shown in green. HRSD DATAFLOW bloom collections in red.

Results:

Phytoplankton composition, including HABs, and non-harmful bloom producers within the James, Elizabeth and Lafayette rivers.

1: Overview: upper James River:

A total of 101 algal taxa were identified in the upper James River. The tidal fresh segments of the river were characterized by a dominance of diatom species. Centric diatoms, in particular were particularly abundant, with *Aulacoseira granuata, Aulacoseira italica*, and *Leptocylindrus danicus* along with pennate diatoms belonging to the genera *Navicula* and *Pleurosigma* making up some of the highest percentages of biomass. Algal species richness and evenness was generally high (Table 2), with a number of sub-dominant and background species, including the diatoms *Cyclotella* spp., *Cylindrotheca closterium, Leptocylindrus minimus,* and *Skeletonema costatum* and *S. potamus*. Common cyanobacteria included filamentous (e.g. *Pseudanabaena limnetica, Aphanizomenon issatshenkoi,* and *Anabaena* spp.) and colonial species (e.g. *Aphanocapsa incerta, Chroococcus dispersus, Merismopedia tenuissima,* and *Microcystis aeruginosa*). Of the chlorophytes, *Actinastrum hantzschii, Pediastrum duplex, Desmodesmus/Scenedesmus* spp., and *Ulothrix* spp. were most common.

2: HABs present in the Upper James:

Common for freshwaters, the HABs present in the Upper James were cyanobacteria. Both the colonial species *Microcystis aeruginosa* and the filamentous *Anabaena circinalis* and *A. spiroides* were present within the river. These species have been associated with the potential to produce algal toxins, and have also been previously noted within the estuary (Marshall et al. 2005). These species were found in background/subdominant densities, with no blooms of these taxa or other cyanobacteria observed during the 2013 collection period. *M. aeruginosa* was present in 7 of the 53 collections in the tidal fresh James, all within the JMSLTF segment, with a maximum density of 2150 cells/ml, and represented between 0.3-4.5% of total algal biomass when present. Microcystin testing was carried out by VCU personnel under the direction of Bukaveckas in the upper James following the protocol of Wood et al. (2013).

Table 2: Phytoplankton characteristics of the James River estuary. Annual mean values and standard error (in parentheses) shown for each parameter.

River Segment	Phytoplankton abundance (cells/ml)	Phytoplankton biomass (µgC/L)	Chlorophyll *a* (µg/L)	Species richness	Species evenness (J')
JMSUTF	1,171 (443)	28 (6)	3.3 (0.5)	6 (0.8)	0.71 (0.05)
JMSLTF	19,156 (5217)	621 (84)	24.3 (2.3)	23 (0.9)	0.61(0.03)
JMSOH	2,737 (1871)	182 (108)	15.9 (8.9)	15 (4.5)	0.70 (0.01)
JMSMH	7,890 (1514)	3,926 (803)	46.9 (6.3)	8 (0.3)	0.46 (0.02)
JMSPH	1,250 (173)	1,117 (436)	12.0 (1.9)	10 (0.4)	0.61 (0.03)
Elizabeth	3,110 (565)	4,327 (1793)	30.0 (6.7)	10 (0.5)	0.42 (0.04)
Lafayette	2,096 (209)	2,795 (543)	35.6 (4.2)	9 (0.2)	0.49 (0.02)

3: Overview: lower James River and the Elizabeth and Lafayette Rivers:

Diatoms and dinoflagellates were the dominant phytoplankton groups in the meso and polyhaline waters of the lower James River estuary. There were 102 phytoplankton taxa encountered in collections from these river segments, although average species richness and evenness per sample was generally low (Table 2). Abundant diatoms included *Skeletonema costatum*, *Pseudo-nitzschia pungens*, *Ceratulina pelagica,* and *Dactyliosolen fragilissimus*. Dinoflagellate populations increased dramatically in mesohaline waters in late winter/early spring, and throughout the region in late summer/early fall as a result of a number of bloom forming species. Non-HAB dinoflagellate bloom species observed this season which are typical for the region included *Heterocapsa triquetra*, *Akashiwo sanguinea*, and *Gymnodinium* spp. (*G. instriatum* et al.).

4: HAB species from the lower James River, and Elizabeth and Lafayette rivers:

Potentially harmful species identified in this section of the estuary during the monitoring season included the dinoflagellates *Alexandrium monilatum, Cochlodinium polykrikoides, Karlodinium veneficum,* and *Prorocentrum minimum. C. polykrikoides* was responsible for a major bloom throughout the region from July to September, while the other species were generally at subdominant or background densities. These taxa have identified as common HABs in the region that have been observed on an annual basis (Marshall and Egerton 2012). *C. polykrikoides* has been associated with fish and shellfish mortality worldwide, including within Chesapeake Bay, with detrimental effects on dissolved oxygen as well as apparent toxicity (Mulholland et al. 2009). In addition to the dinoflagellates, HAB raphidophytes *Chatonella subsalsa* and *Heterosigma akashiwo* were present in the Elizabeth and Lafayette Rivers, albeit at

background densities. Diatoms belonging to the genus *Pseudo-nitzschia* including *P. pungens*, were identified in the estuary as well. These taxa have been associated with the production of the toxin domic acid in parts of the world, including Chesapeake Bay (Thessen and Stoecker 2008). Whether this taxa represents a threat to Chesapeake Bay is debatable, but they are included here and elsewhere as HAB species due to their potential to produce toxins (Marshall et al. 2005). *P. pungens* is a common component to the mesohaline waters of Chesapeake Bay and its tributaries and has been observed annually during routine long-term monitoring. There have been no *P. pungens* related HABs in Chesapeake Bay, and quantifiable domoic acid concentrations of this species have not been detected in monoculture (Thessen and Stoecker 2008).

Characterization of phytoplankton abundance and biomass in upper James during 2013

There were no distinct algal blooms in the upper James River segments. Total algal biomass fluctuated during the monitoring season at both the upper and lower tidal fresh stations, with lowest concentrations in July and highest in September and October (Figure 3). Biomass at the upper tidal fresh station (TF5.3) was lowest in the river (Table 2), ranging from 1.3-127 µgC/L. Phytoplankton abundance was generally sparse, and varied from 20-9,020 cells/ml. Diatoms made up at average of 81% of algal biomass at TF5.3, with cyanobacteria and chlorophytes contributing 11 and 7% respectively.

Twenty-four miles downstream, at the lower tidal fresh station TF5.5, phytoplankton abundance and biomass was higher by more than an order of magnitude (Table 2). Cell density ranged from 980-128,860 cells/ml, including the highest abundance values in the James, while algal biomass ranged from 126-1526 µgC/L. Diatoms, particularly centrics, contributed the highest amount of biomass, an average of 81%. A diverse group of chlorophytes (7%), cyanobacteria (5%), dinoflagellates (4%) and euglenoids (3%) contributed the remainder of algal biomass. Species composition was generally similar throughout the monitoring season regardless of total abundance/biomass and varied little in comparison to Chl *a* at both stations (Figure 4). Even at the highest Chl *a* level measured in this segment (48.7 µg/L), algal species richness was highest and species evenness remained relatively high, with a similar composition to lower biomass collections taken from the same segment (Table 3). As a whole, within the upper James River, there is a significant positive correlation between Chl *a* and algal species richness, and no significant effect on species evenness (Figure 5).

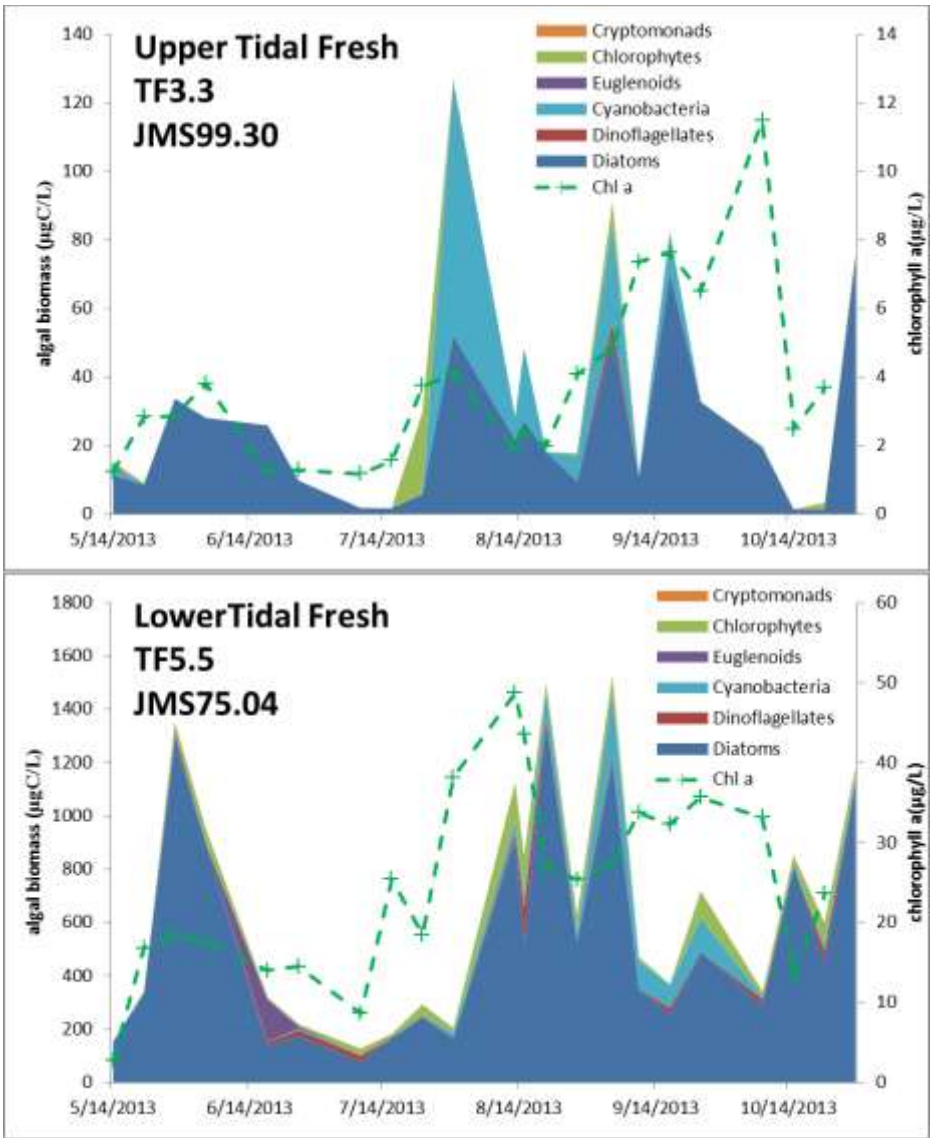

Figure 3: Weekly phytoplankton biomass estimates and chlorophyll concentrations at two stations in the Upper James River. Note difference in Y axes between TF5.3 and TF5.5.

Table 3: Average seasonal algal biomass per taxonomic group per Chlorophyll *a* level for each river segment in the Upper James River

River Segment	Season	Chl *a* (µg/L)	Mean algal biomass (µgC/L)							
			Diatoms	Dinoflagellates	Cyanobacteria	Euglenophytes	Chlorophytes	Cryptomonads	Raphidophytes	**Total Biomass**
James River Upper Tidal Fresh **JMSUTF**	**Spring** March 1- May 31, 2013	<6	17.9	0.0	0.9	0.0	0.2	0.4	0.0	**19.3**
		6-12								
		12-24								
		24-36								
		36-48								
		48-100								
		>100								
	Summer July 1- Sept. 30, 2013	<6	16.3	0.3	10.6	0.0	2.5	0.0	0.0	**29.7**
		6-12	37.9	0.0	4.1	0.0	0.3	0.0	0.0	**42.3**
		12-24								
		24-36								
		36-48								
		48-100								
		>100								
James River Lower Tidal Fresh **JMSLTF**	**Spring** March 1- May 31, 2013	<6	152.5	0.0	0.6	0.0	0.7	0.0	0.0	**153.8**
		6-12								
		12-24	806.3	0.0	3.1	15.3	22.6	0.3	0.0	**847.5**
		24-36								
		36-48								
		48-100								
		>100								
	Summer July 1- Sept. 30, 2013	<6								
		6-12	68.1	10.6	3.3	0.0	15.7	0.8	0.0	**98.6**
		12-24	557.9	17.8	17.0	0.0	22.2	0.3	0.0	**615.2**
		24-36	598.8	9.9	94.5	5.7	37.5	0.3	0.0	**746.8**
		36-48	346.7	63.8	31.2	4.0	80.5	0.3	0.0	**526.5**
		48-100	913.6	21.3	44.1	0.0	147.3	0.0	0.0	**1126.3**
		>100								

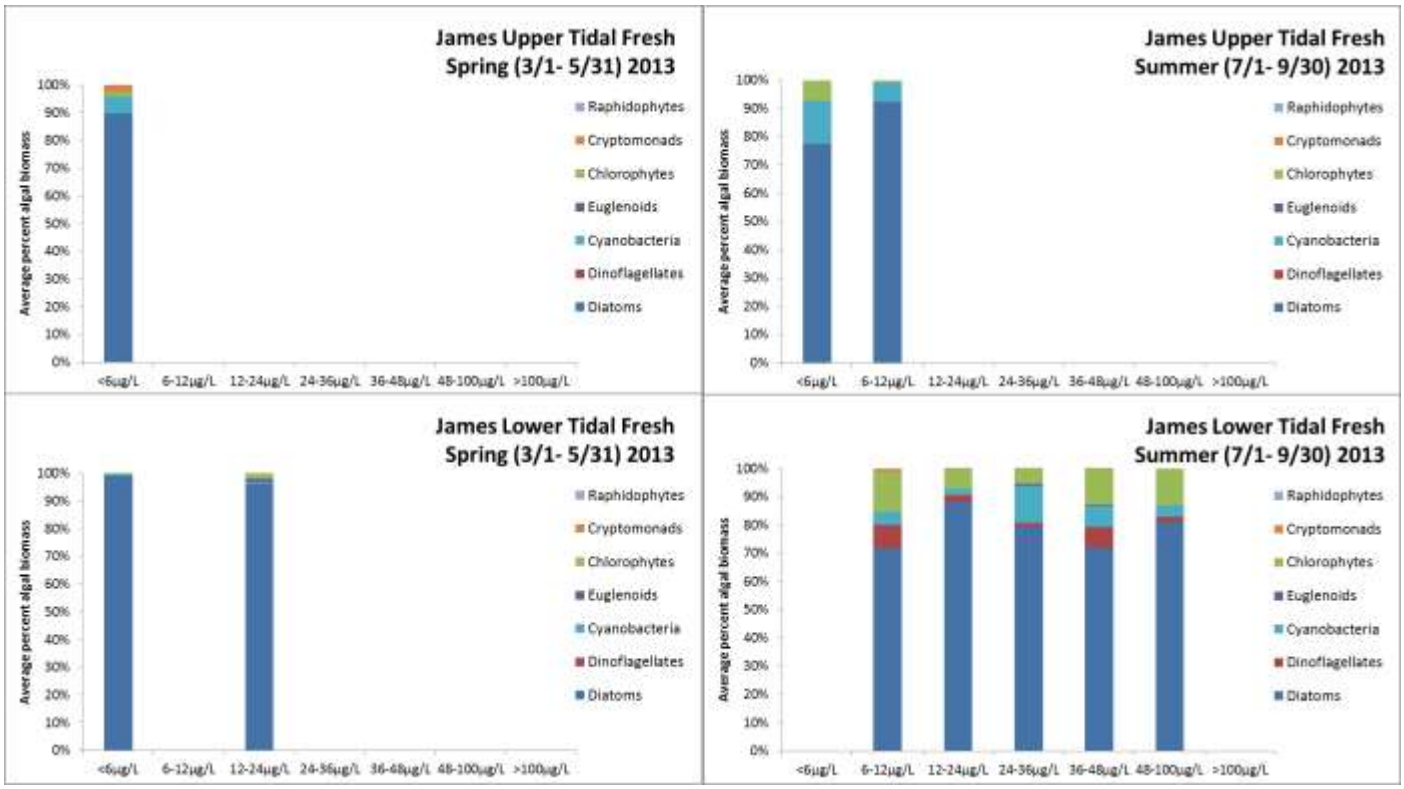

Figure 4: Relative taxonomic algal biomass compostion in relation to chlorophyll concentration at stations TF5.3 (upper) and TF5.5 (lower) in spring (left) and summer (right) 2013.

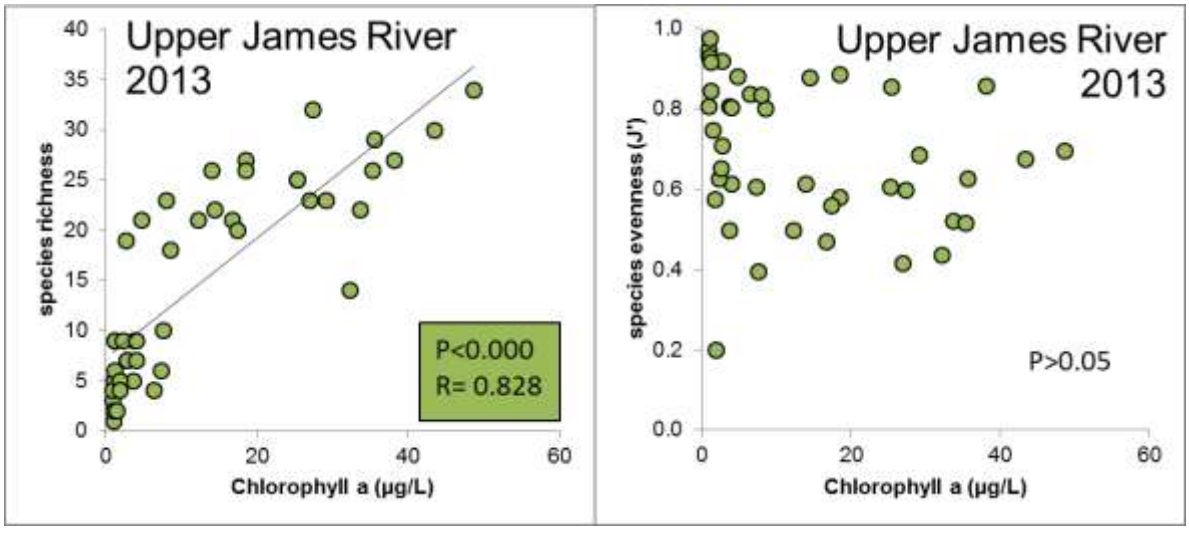

Figure 5: Relationships between chlorophyll *a* and phytoplankton diversity; species richness (left) and species evenness (right).

Characterization of phytoplankton abundance and biomass in lower James during 2013

As in previous years, two major dinoflagellates blooms occurred in the meso-and polyhaline regions of the James in 2013 (Figure 6). The spring bloom dominated by the non-HAB species *Heterocapsa triquetra* persisted for approximately 11 weeks in the mesohaline waters during February, March and April. Maximum bloom development was associated with chlorophyll concentrations in excess of the manufacturer's upper limit (>400µg/L), and a maximum *H. triquetra* cell densities of 178,070 cells/ml. Biomass estimates reached a maximum of 6.7 $x10^4$ µgC/L. While highest concentrations were observed in the mesohaline region, *H. triquetra* was observed throughout the lower James River estuary in 2013 including the Elizabeth and Lafayette Rivers (Figure 7).

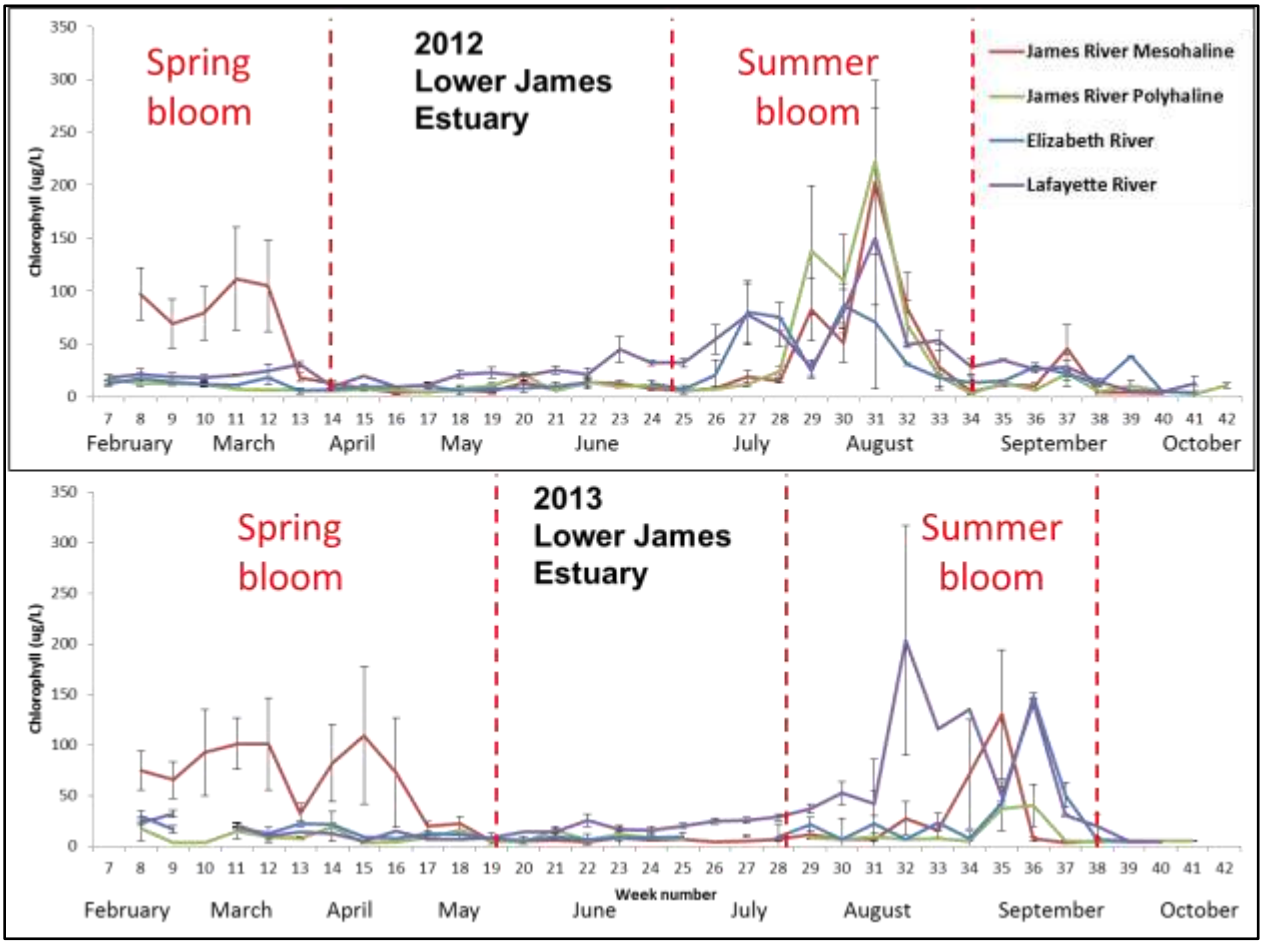

Figure 6: Weekly mean chlorophyll concentrations in the meso- and polyhaline James, Elizabeth Rivers in 2012 (top) and 2013 bottom. Seasonal dinoflagellate blooms occurred in spring (Feb-April) and Summer (July-September).

The summer/autumn dinoflagellate bloom had a duration of approximately 8 weeks in 2013, spanning July, August and September. Over this period of time, there was a succession of dinoflagellate species with elevated densities, with the HAB species *Cochlodinium polykrikoides* being the dominant taxon. *C. polykrikoides* densities during the bloom had a maximum density of 22, 880 cells/ml. During this time period, Chl *a* once again exceeded the manufacturer's threshold (>400µg/L), and biomass estimates reached a maximum of 7.8 $x10^4$ µgC/L. *C. polykrikoides* was present across the estuary, with highest concentrations in the Lafayette, Elizabeth and mesohaline James River segments (Figure 8). Prior to the larger *C. polykrikoides* bloom, there were elevated concentrations of *Gymnodinium* spp. (Figure 9) and *Akashiwo sanguinea* (Figure 10). In the case of *A. sanguinea* blooms, Chl *a* concentrations exceeded 100 µg/L. This marks the first time in this study (2011, 2012, 2013) that a species other than *H. triquetra* and *C. polykrikoides* is responsible for a bloom of this magnitude.

Both spring and summer blooms in the lower James were associated with very different phytoplankton composition compared to non-bloom periods. Elevated Chl *a* , generally over 12 µg/L was associated with dinoflagellate biomass, most often a bloom of *H. triquetra* or *C. polykrikoides* (Figure 11). Within these communities, dinoflagellates were dominant, and there were lower levels of species diversity (especially species evenness) (Figure 12). Higher concentrations (ie. >36 µg/L) represented algal communities were up to 99% of the biomass was from a single blooms species (*H. triquetra* or *C.polykrikoides*) (Figure 11, Table 4).

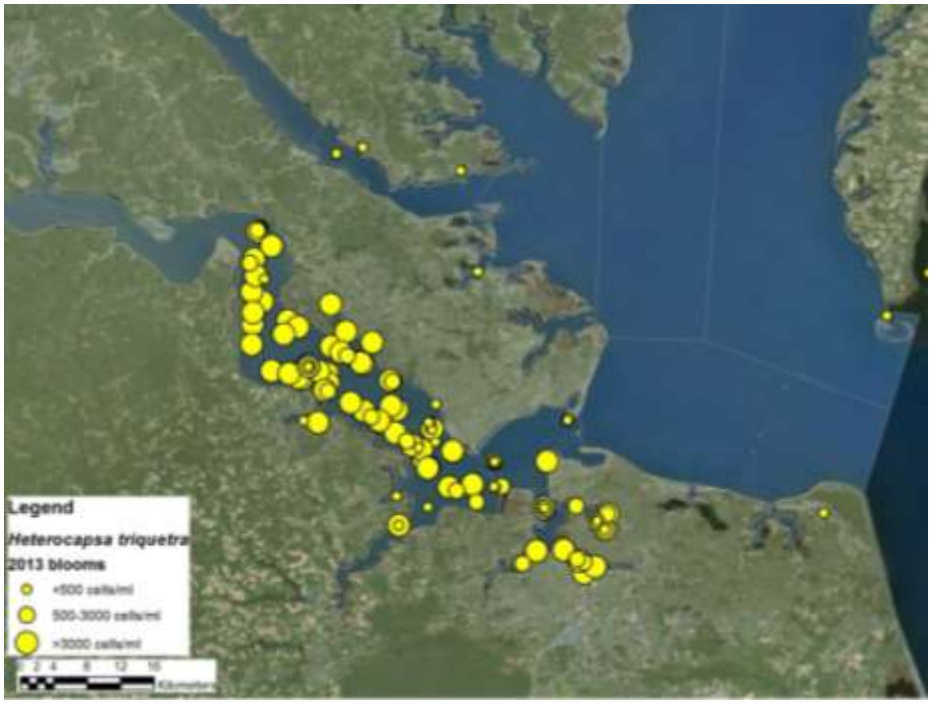

Figure 7: 2013 distribution of *Heterocapsa triquetra* in lower James River and surrounding waters.

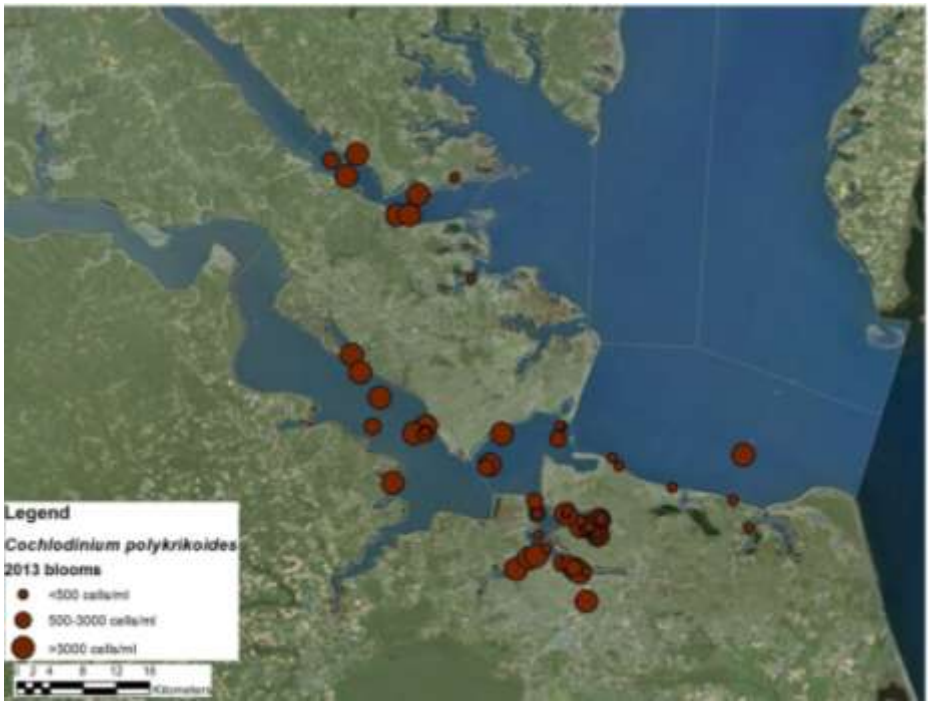

Figure 8: 2013 distribution of *Cochlodinium polykrikoides* in lower James River and surrounding waters.

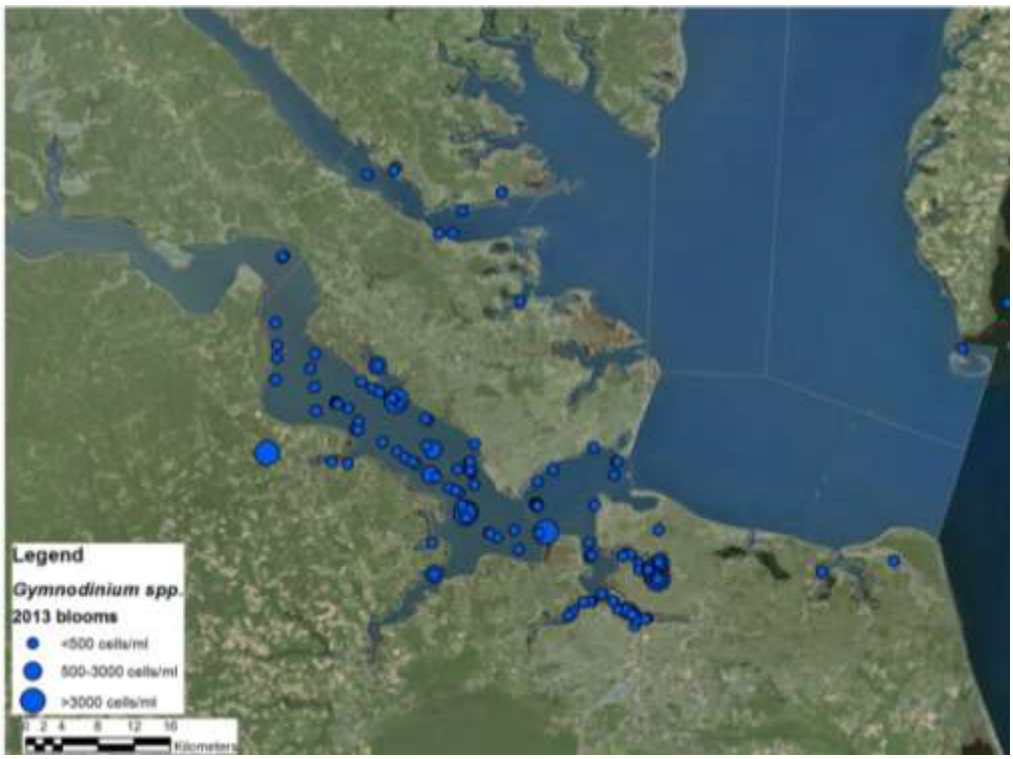

Figure 9: 2013 distribution of *Gymnodinium* spp. in lower James River and surrounding waters.

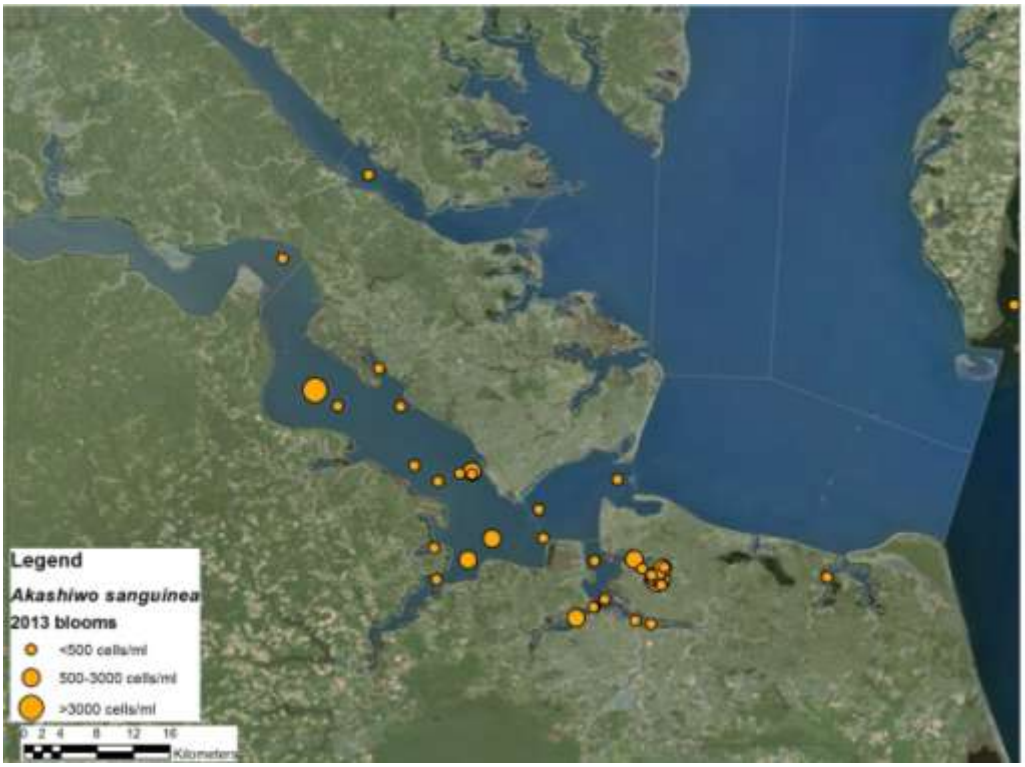

Figure 10: 2013 distribution of *Akashiwo sanguinea* in lower James River and surrounding waters.

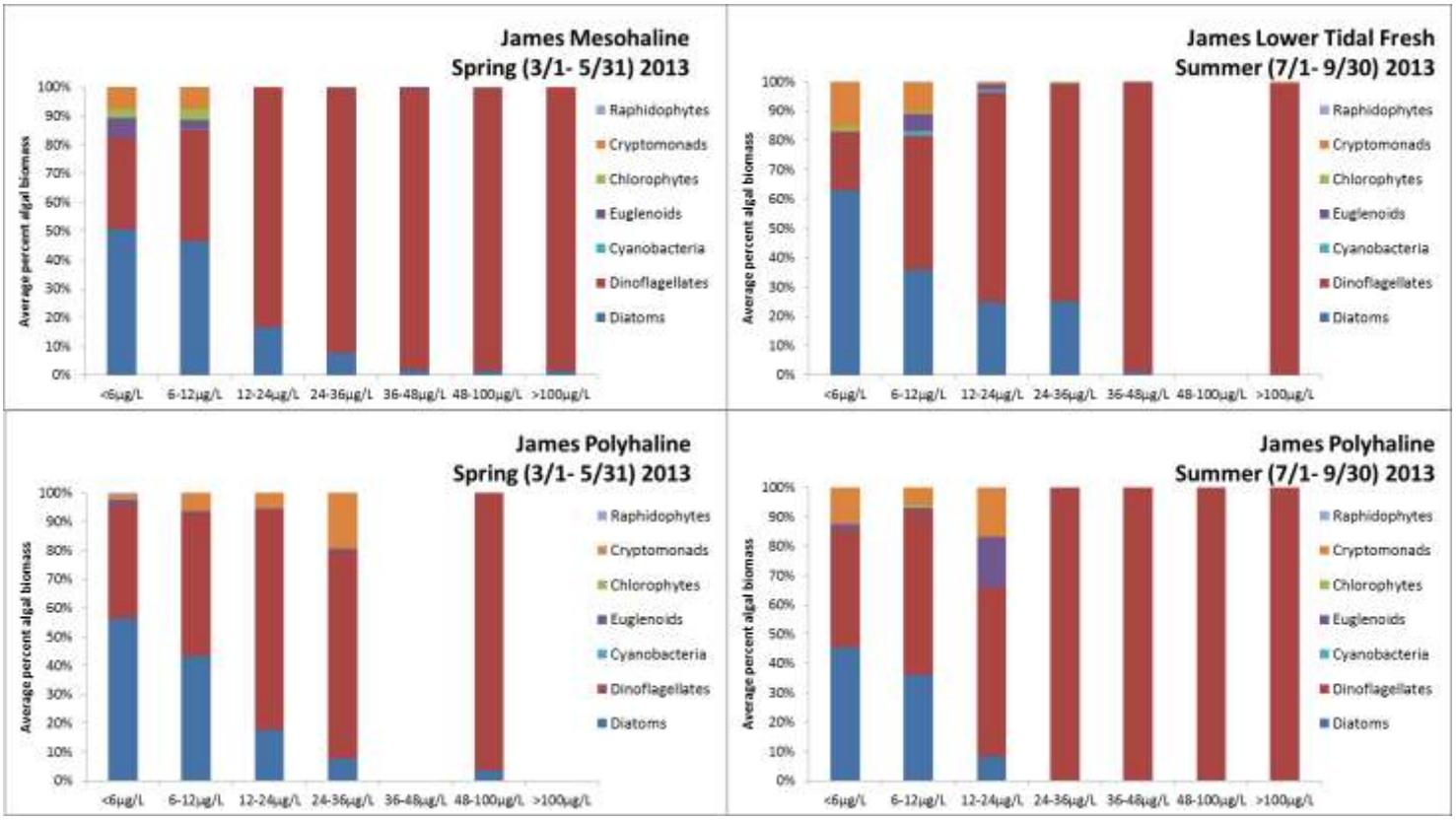

Figure 11: Relative taxonomic algal biomass compostion in relation to chlorophyll concentration in segment JMSMH (upper) and JMSPH (lower) in spring (left) and summer (right) 2013.

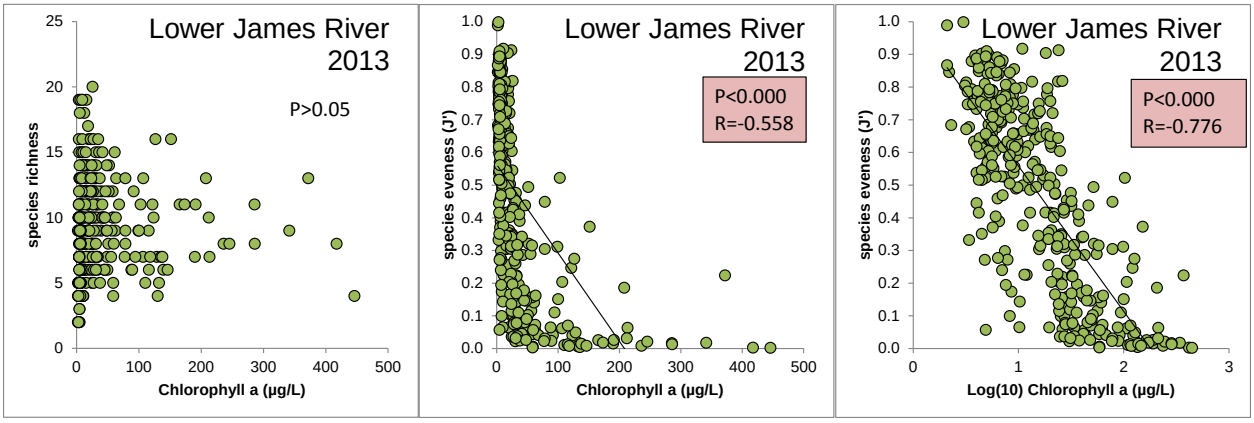

Figure 12 : Relationships between chlorophyll *a* and phytoplankton diversity in lower James River; species richness (left) and species evenness (middle and right). The relationship between Chl *a* and evenness is best described as a negative logarithmic relationship (right).

Table 4: Average seasonal algal biomass per taxonomic group per Chlorophyll *a* level for each river segment in the Lower James River

River Segment	Season	Chl *a* (µg/L)	Mean algal biomass (µgC/L)							
			Diatoms	Dinoflagellates	Cyanobacteria	Euglenophytes	Chlorophytes	Cryptomonads	Raphidophytes	**Total Biomass**
James River Mesohaline **JMSMH**	**Spring** March 1- May 31, 2013	<6	25.1	20.2	0.0	3.4	1.1	1.7	0.0	**51.6**
		6-12	62.3	112.7	0.2	2.7	4.5	4.0	0.0	**186.2**
		12-24	82.5	323.3	0.0	0.0	0.0	0.9	0.0	**406.7**
		24-36	57.6	1,283.6	0.0	5.7	0.1	2.5	0.0	**1,349.5**
		36-48	83.4	3,412.3	0.0	36.2	0.0	1.7	0.0	**3,533.6**
		48-100	68.9	5,520.4	0.0	9.8	0.1	3.9	0.0	**5,603.1**
		>100	82.6	19,023.3	0.1	1.7	0.2	0.7	0.0	**19,108.5**
	Summer July 1- Sept. 30, 2013	<6	24.6	14.6	0.0	0.0	1.0	4.3	0.0	**44.6**
		6-12	40.2	99.9	0.4	2.9	0.7	8.4	1.1	**153.7**
		12-24	49.6	509.6	1.8	4.0	0.0	3.9	0.0	**568.9**
		24-36	129.0	2,635.2	0.4	2.0	2.8	2.1	0.0	**2,771.6**
		36-48	13.6	1,375.5	0.0	0.0	0.0	0.0	0.0	**1,389.1**
		48-100								
		>100	10.7	34,895.1	0.0	2.0	0.0	27.0	0.0	**34,934.8**
James River Polyhaline **JMSPH**	**Spring** March 1- May 31, 2013	<6	88.4	88.5	0.0	1.7	0.0	2.1	0.5	**181.3**
		6-12	286.4	253.5	0.0	1.8	0.0	8.3	0.0	**550.0**
		12-24	97.0	537.6	0.0	4.8	0.0	10.5	0.0	**650.0**
		24-36	44.7	558.6	0.0	2.7	0.0	44.9	0.0	**650.8**
		36-48								
		48-100	288.2	8,117.8	0.0	0.0	0.0	0.0	0.0	**8,406.0**
		>100								
	Summer July 1- Sept. 30, 2013	<6	25.8	81.5	0.0	1.9	0.0	1.8	0.0	**110.9**
		6-12	49.6	79.9	0.0	1.6	1.0	4.2	0.0	**136.3**
		12-24	8.4	468.5	0.0	12.1	0.0	12.5	0.0	**501.4**
		24-36	1.0	3,325.6	0.0	0.0	0.0	0.0	0.0	**3,326.6**
		36-48	6.5	8,518.9	0.0	0.0	0.0	0.0	0.0	**8,525.5**
		48-100	9.9	9,557.0	0.0	0.0	0.0	0.3	0.0	**9,567.2**
		>100	1.5	31,506.5	0.0	0.0	0.0	0.6	0.0	**31,508.6**

The initiation, composition, and progressions of bloom development in the James River in 2013 in comparison to blooms occurring in 2011 and 2012.

Within the upper James River, no distinct algal blooms per se occurred in any of the years of the monitoring program. The flora has remained relatively consistent during the study from year to year, with a dominance of diatoms (*Aulacoseira granulata* et al.), and a diverse number of other taxa that increases generally overall during the summer months. However, there was variation between years in the overall algal densities/biomass/ Chl *a*, particularly in the lower tidal fresh segment. In 2012, the maximum chlorophyll at station TF5.5 reached 66.72µg/L, 37% higher than the highest measured in 2013 (48.7 µg/L). In 2012 during the DEQ spring and summer regulation periods (3/1-5/31 and 7/1-9/30), Chl *a* concentrations at TF5.5 were at or above 36 µg/L for 6 weeks, opposed to just 2 weeks in 2013 (Table 5). There was little difference in composition, but algal biomass was also lower in 2013 than 2012. At TF5.5, the 2013 highest biomass was only 1224 µgC/L compared to 2025 µgC/L the year before.

In the lower James, there also were differences in algal development from past years, specific to the blooms within the region. The spring bloom of *Heterocapsa triquetra* began in February in 2013, similar to 2012, and 7 weeks earlier than observed in 2011. It should be noted that this was the first week of sampling in each case, and initiation could have occurred prior to sampling. Maximum bloom concentrations of *H. triquetra*, with Chl *a* concentrations of at least 100 µg/L and densities 15,000 cells/ml or greater were present for 8 weeks between February 19 and April 17, 2013. These same conditions were present for 5 weeks in 2012 (Feb. 21-Mar. 20) and only 2 weeks in 2011(Apr 6-13). While the duration of the *H. triquetra* bloom was longer this year the magnitude was comparable, with 178,070 cells/ml in 2013 and 191,200 cells/ml in 2012. In both cases, the maximum chlorophyll measurements were above the manufacturer's limit. The highest density observed in 2011 was only 65,000 cells/ml. In all years, the densest portions of the bloom appear to be largely constrained within the mesohaline segment, initiating in the Burwell Bay vicinity, with highest concentrations developing near the Warwick River.

Several studies have documented the initiation and transport of the summer/autumn HAB *Cochlodinium polykrikoides* (ie. Marshall and Egerton 2009, Morse et al. 2011, Egerton et al. 2012). In 2013, bloom concentrations of $\geq$ 3000 cells/ml were present for 5 weeks between August 6 and September 9, compared to 7 weeks (June 26-Aug 8) in 2012 and 5 weeks in 2011(July 27-Aug 23). In addition to a shorter duration, the magnitude of the *C. polykrikoides*

bloom was also reduced from 2012. Maximum concentrations in 2013 were 22, 880 cells/ml, down from a maximum of 75,780 cells/ml last year. Spatially, the *C. polykrikoides* bloom was much more isolated to the Lafayette and Elizabeth River than 2012 with much lower concentrations in the meso and polyhaline James (Figure 6, Table 5) compared to last year.

Table 5: Summary of maximum Chl *a* concentrations measured in each river segment during spring and summer 2012/2013.

Season/ Year	River Segment	# weeks sampled	Number of weeks with chlorophyll levels were present greater than:					
			>6µg/L	>12µg/L	>24µg/L	>36µg/L	>48µg/L	>100µg/L
Spring 2012	UTF	4	0	0	0	0	0	0
	LTF	4	4	3	2	2	1	0
	MH	8	8	5	3	2	2	2
	PH	9	7	1	0	0	0	0
	ER	8	6	2	0	0	0	0
	LAF	9	9	8	3	0	0	0
Summer 2012	UTF	11	5	1	0	0	0	0
	LTF	11	10	10	9	4	1	0
	MH	9	9	8	6	6	5	4
	PH	9	8	8	6	5	5	4
	ER	9	9	9	6	5	4	4
	LAF	9	9	9	9	9	7	4
Spring 2013	UTF	0						
	LTF	2	2	2	0	0	0	0
	MH	10	12	9	9	9	8	6
	PH	10	8	5	3	1	1	0
	ER	9	11	5	1	0	0	0
	LAF	9	11	7	2	0	0	0
Summer 2013	UTF	11	3	0	0	0	0	0
	LTF	11	11	10	9	2	1	0
	MH	12	9	6	5	4	3	3
	PH	11	9	3	2	2	2	1
	ER	12	11	6	6	3	3	1
	LAF	12	12	12	12	10	9	5

Relationships between seasonal phytoplankton and biomass and chlorophyll levels in the James, Elizabeth, and Lafayette rivers.

Cell abundance alone may not indicate the major contributors to biomass and chlorophyll present at these river sites. Many of the cyanobacteria noted at high abundance levels consist of cells less than 2 microns in size, and although their accumulative abundance numbers may be high, their total biomass and the chlorophyll content in their cells may be considerably less than

less abundant (but larger sized) diatoms or dinoflagellates in the water column. For this reason, algal biomass values are calculated based on cell biovolume and compared to measured chlorophyll values.

In the upper James, there is a strong positive linear correlation between Chl *a* and phytoplankton biomass ($p<0.000$, $R=0.730$) (Figure 13). In this section, diatoms account for an average of 83% of the biomass, with cyanobacteria and chlorophytes making up 7 and 6% respectively. In addition to total biomass, Chl *a* is also significantly positively correlated with diatoms, dinoflagellates, chlorophytes and cyanobacteria (Figure13).

In the lower James River estuary, inclusive of the Elizabeth and Lafayette there is an even stronger positive linear correlation between Chl *a* and phytoplankton biomass ($p<0.000$, $R=0.813$) (Figure 14). However, unlike the overall increase in the phytoplankton community found in the upper James, this signal is driven almost entirely by the dinoflagellate blooms in the region. Dinoflagellate biomass is highly correlated with Chl *a* (Figure 14), but there is no significant relationship with any of the other major taxonomic groups (diatoms $p=0.273$, cyanobacteria $p=0.493$, chlorophytes $p=0.619$, cryptomonads $p=0.344$, euglenoids $p=0.194$, raphidophytes $p=0.704$). These are similar results as those found in 2012, with dinoflagellates making up the vast majority of the Chl *a* signal, although this year it appeared that a larger number of dinoflagellate species were responsible.

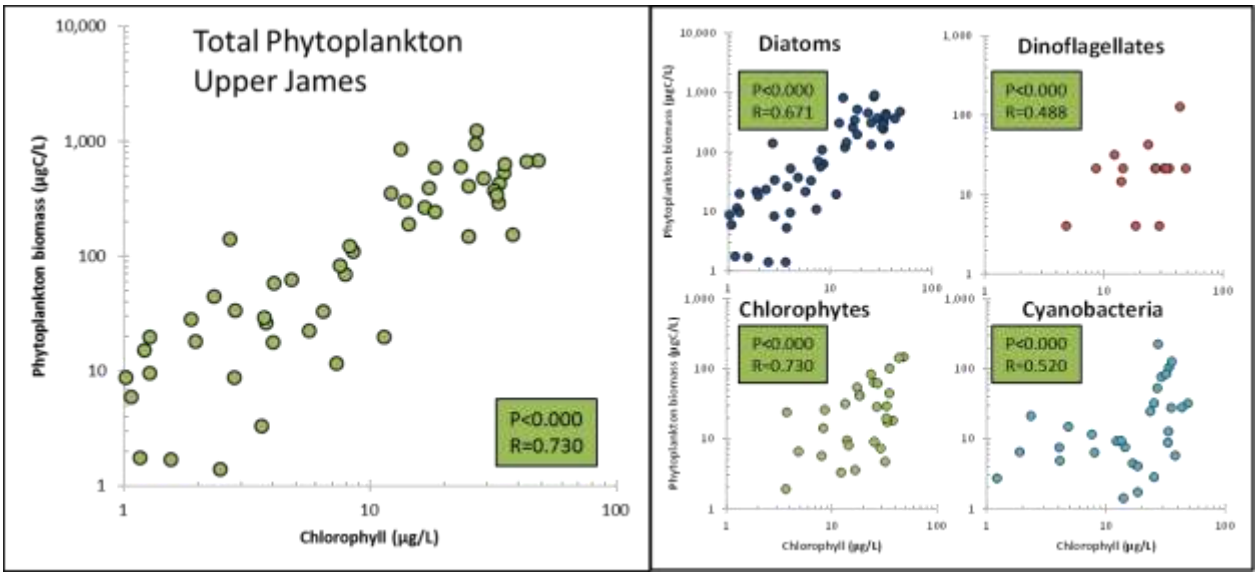

Figure 13: Correlations between tidal fresh and oligohaline algal biomass and Chl *a* concentrations.

Table 6: Summary statistics of Chl *a* relationships to phytoplankton biomass for each taxonomic group and total phytoplankton by river segment and season. Only significant correlations ($p<0.05$) shown. Summary statistics include significance (p), Pearson's Correlation coefficient (R), slope (m) of linear relationship between (x) Chl *a* (µg/L) and (y) biomass (µgC/L), and y-intercept (b). *Table shown on next page.*

Season	River Segment	Sample # (n)	Diatoms	Dinoflagellates	Cyanobacteria	Euglenophytes	Chlorophytes	Cryptomonads	Raphidophytes	**Total Biomass**
Spring March 1-May 31, 2013	UTF	3								
	LTF	3								
	OH	3								
	MH	76		p <0.000 R= 0.87 m= 97.3 b= -1286.8						p <0.000 R= 0.87 m= 96.8 b= -1111.7
	PH	30		p <0.000 R= 0.77 m= 114.7 b= -697.3						p <0.000 R= 0.75 m= 115.4 b= -529.9
	ER	14		p <0.000 R= 0.89 m= 115.2 b= -819.8						p <0.000 R= 0.86 m= 146.1 b= -943.8
	LAF	18		p <0.000 R= 0.80 m= 313.5 b= -3281.8						p <0.000 R= 0.79 m= 311.3 b= -3102
Summer July 1- Sept. 30, 2013	UTF	16	p= 0.006 R= 0.65 m= 5.8 b= 1.6							p= 0.008 R= 0.64 m= 6.8 b= 3.6
	LTF	16					p= 0.008 R= 0.64 m= 2.6 b= -27.2			
	OH	3								
	MH	52		p <0.000 R= 0.927 m= 204.3 b= -2176.5						p <0.000 R= 0.928 m= 201.3 b= -1711.9
	PH	30		p <0.000 R= 0.989 m= 267.5 b= -1971.5						p <0.000 R= 0.988 m= 266.4 b= -1880
	ER	27		p <0.000 R= 0.980 m= 268.1 b= -4005.2						p <0.000 R= 0.980 m= 267.2 b= -3861.7
	LAF	68		p <0.000 R= 0.768 m= 105.4 b= -1030.2		p= 0.041 R= 0.248 m= 0.06 b= 2.7				p <0.000 R= 0.768 m= 105.4 b= -983.3

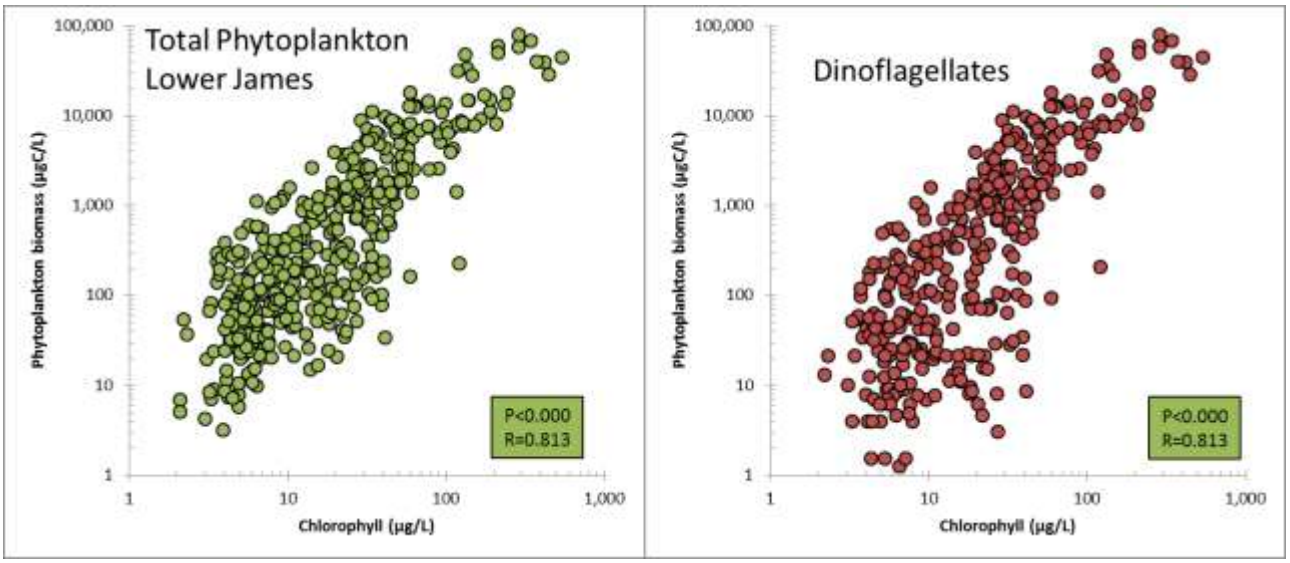

Figure 14: Correlations between meso-polyhaline algal biomass and Chl *a* concentrations.

Daily sampling in Lafayette River

To further capture the spatial and temporal variability of rapidly developing algal blooms, two stations were sampled daily by Dr. Filippino (ODU OEAS) beginning 6/3/2013. As previous work had identified the Lafayette River as the likely initiation point of the *C. polykrikoides* and other dinoflagellate blooms (Morse et al. 2011, Egerton et al. 2014), two locations within the river were chosen, station AC in the headwaters, and NYCC near the river mouth (Figure 2) to track the development and transport of the bloom. The time period of the study was based on 2012 results, when *C. polykrikoides* was first observed in the 3rd week of June, to capture conditions and community dynamics leading up to the bloom. However, in 2013 *C. polykrikoides* was not observed in these collections until 7/18 (Fig 15). Over the course of ~ 2 weeks, *C. polykrikoides* densities increased upstream from 20 cells/ml to over 2000 cells/ml, before reaching a maximum concentration of ~13,000 cells/ml at station AC. During this time period Chl *a* concentrations increased 10 fold from ~50µg/L to 540 µg/L. There was a strong positive correlation between Chl *a* concentrations and phytoplankton biomass estimates ($p<0.001$, $R= 0.983$). Downstream, at station NYCC, *C. polykrikoides* densities were much lower, reaching a maximum during this study of 660 cells/ml on August 12, 2013. Chlorophyll concentrations were subsequently also lower, with a maximum of just 52 µg/L. Prior to the *C. polykrikoides* bloom, there were a dominance of diatom taxa, particularly downstream, followed

by an increased abundance of other dinoflagellates beginning around 6/25/13. Prominent sub-dominant taxa included *Gymnodinium instriatum* and *Akashiwo sanguinea*, which were associated with Chl *a* concentrations up to 61 µg/L.

Station AC was also sampled every two hours over a 24hr period during the height of the *C. polykrikoides* bloom by Filippino, on August 9. During this period, *C. polykrikoides* accounted for up to 98% of the total phytoplankton biomass. Cell densities of *C. polykrikoides* at the site varied from 60-5460 cells/ml; a difference of almost 2 orders of magnitude within 24 hrs. This variability was reflected in the range in total phytoplankton biomass, from 283 µgC/L to 19,147 µgC/L (Figure B). Greater cell abundance/biomass occurred during daylight hours, coinciding with both low-tide and incoming high-tide, with lowest concentrations during outgoing tide and at night. In addition to the implications of tidal transport, and possible vertical migration of the dinoflagellates in response to light, these results also exemplify the temporal variability associated with HABs. This variability would need to be included or at least acknowledged in any mathematical model of bloom dynamics.

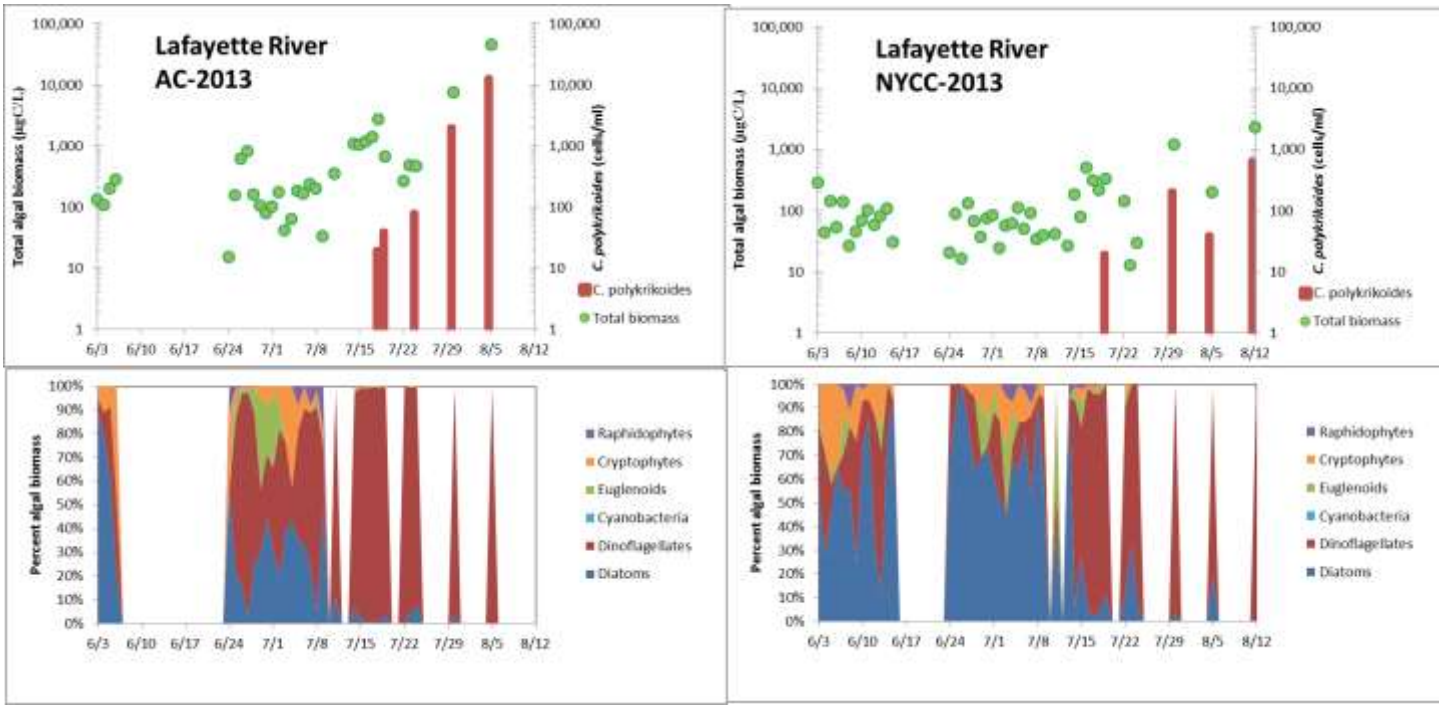

Figure 15: Time series of Lafayette River sampling during June-August 2013 at upstream site AC (left) and downstream site NYCC (right). Total algal biomass estimates and *Cochlodinium polykrikoides* densities shown in top panels, and relative biomass of the phytoplankton community in bottom panels.

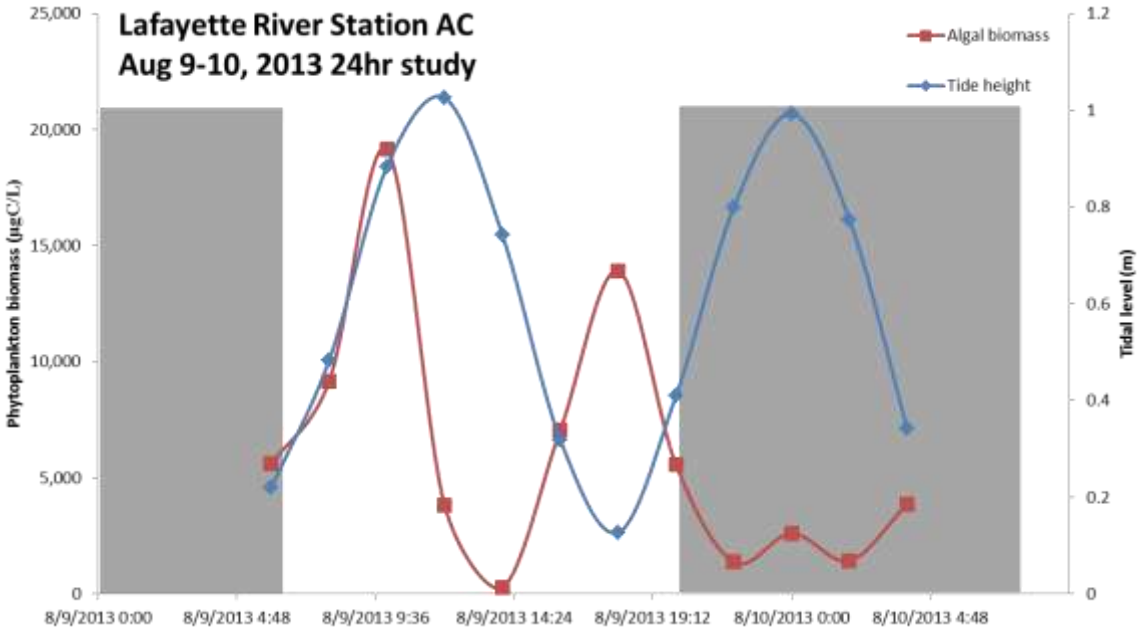

Figure 16: Time series of 24hr study of Lafayette Station AC during bloom showing total algal biomass and tidal height. *Cochlodinium polykrikoides* contributed up to 98% of biomass at this time. Grey bars represent night (sunset-sunrise).

Summary statements

1. The James River supports a diverse phytoplankton community represented by over 150 algal taxa, with great variability in composition and biomass both seasonal and spatially in response changing environmental conditions.
2. Analysis of a total of 503 samples this year identified the major contributors of algal biomass in the James River estuaries. In the upper James River an average of 83% of the biomass belonged to diatoms, with cyanobacteria and chlorophytes contributing 7 and 6% respectively. In the lower James estuary, dinoflagellates were dominant; making up an average of 64% of the biomass, with diatoms contributing 27%. Biomass estimates of the dominant groups were significantly positively correlated with Chl *a* concentrations in both regions and varied by season and segment.
3. Nine HAB species were observed in the estuary over the course of the 2013 monitoring study. In the tidal freshwater upper James, these included the cyanobacteria *Anabaena circinalis, A. spiroides* and *Microcystis aeruginosa* . Cell concentrations were well below established VDH/DEQ levels of health concern. In the lower estuary, HAB species included the dinoflagellates *Alexandrium monilatum, Cochlodinium*

polykrikoides, *Karlodinium veneficum*, and *Prorocentrum minimum*, the diatom *Pseudo-nitzschia pungens* and the raphidophytes *Chatonella subsalsa* and *Heterosigma akashiwo*.

4. The upper James River was characterized by the highest diversity of phytoplankton taxa in the estuary, with increased biomass and Chl *a* associated with a general increase in the overall algal community, not specific to a bloom of a single species or group. There was no marked linkage between Chl *a* concentration and a change to the makeup of the phytoplankton community.
5. Algal biomass and Chl *a* concentrations were lower in the upper James River in 2013 than in 2012, both in magnitude and duration. Chlorophyll *a* concentrations in the tidal fresh were >36µg/L for only 2 weeks in 2013, down from 9 weeks in 2012. Maximum biomass values and Chl *a* concentrations were reduced as well.
6. Chlorophyll and phytoplankton biomass concentrations were highest in the meso and polyhaline waters of the estuary, reaching values >400µg Chl *a*/L and >78,000µg C/L. These values were associated with two seasonal near monospecific dinoflagellate blooms in the lower James River where single species accounted for up to 99% of the total algal biomass.
7. Mesohaline waters experienced a spring bloom of the non-HAB dinoflagellate *H. triquetra* for approximately 8 weeks, reaching a maximum density of ~1.8 $x10^5$ cells/ml. This is a longer duration than observed in 2012 (5 weeks) and 2011 (2 weeks), and a comparable magnitude (1.9 $x10^5$ cells/ml in 2012). During the bloom Chl *a* concentrations exceeded 400 µg/L.
8. The summer dinoflagellate bloom was dominated by *C. polykrikoides*, but also high concentrations of *A. sanguinea* and other dinoflagellates. Bloom development was found throughout the Lafayette and Elizabeth Rivers, with reduced concentrations in the mesohaline and polyhaline James River from last year. Bloom conditions lasted for ~5 weeks compared to 7 weeks in 2012 and 5 weeks in 2011. Maximum densities were also reduced from last year.
9. Elevated Chl *a* concentrations in waters of the lower James River estuary were strongly associated with algal blooms, and a significantly different phytoplankton composition than lower Chl *a* values. High Chl *a* concentrations (>36µg/L) were virtually always associated with a bloom where a single species accounted for up to 99% of the biomass.

Even at lower concentrations, Chl *a* was negatively correlated to species diversity in the lower James River.

10. Weekly, daily and hourly sampling further revealed the temporal variability intrinsic to HABs in a tidal estuary. Phytoplankton biomass at a single site varied by almost 2 orders of magnitude in a 24 hour period, and suggests both tidal movement and light-cued vertical migration play important roles in HAB dynamics that should be considered when modeling these organisms.

References

Boyer, J. N., Kelble, C. R., Ortner, P. B., & Rudnick, D. T. 2009. Phytoplankton bloom status: Chlorophyll *a* biomass as an indicator of water quality condition in the southern estuaries of Florida, USA. E*cological Indicators*, *9*(6), S56-S67.

Dauer, D. M., H. G. Marshall, J. R. Donat, M. F. Lane, S. C. Doughten, and C. Johnson. 2012. Current status and long-term trends in water quality and living resources in the Virginia tributaries and Chesapeake Bay mainstem from 1985 through 2011. Report to Virginia Department of Environmental Quality. Old Dominion University, Norfolk, Virginia.

Egerton, T. A. 2013. Investigations of phytoplankton diversity in Chesapeake Bay. Ph.D. dissertation. Old Dominion University. Norfolk, Virginia.

Egerton, T. A., Morse, R. E., Marshall, H. G., & Mulholland, M. R. 2014. Emergence of Algal Blooms: The Effects of Short-Term Variability in Water Quality on Phytoplankton Abundance, Diversity, and Community Composition in a Tidal Estuary. *Microorganisms*, *2*(1), 33-57.

Egerton, T.A., W. Hunley, and H.G. Marshall. 2012. Integration of microscopy and underway chlorophyll mapping for monitoring algal bloom development. Proceedings. OCEANS 12 Conference, Hampton Roads, Virginia.

Filstrup, C. T., Hillebrand, H., Heathcote, A. J., Harpole, W. S., & Downing, J. A. (2014). Cyanobacteria dominance influences resource use efficiency and community turnover in phytoplankton and zooplankton communities. *Ecology Letters* 17, 464-474.

Harding Jr, L. W., R. A. Batiuk, T. R. Fisher, C. L. Gallegos, T. C. Malone, W. D. Miller, M. R. Mulholland, H. W. Paerl, E. S. Perry, and P. Tango. 2014. Scientific Bases for Numerical Chlorophyll Criteria in Chesapeake Bay. *Estuaries and Coasts* 37, 134-148.

Korhonen, J. J., Wang, J., & Soininen, J. 2011. Productivity-diversity relationships in lake plankton communities. *PloS one*, 6(8), e22041.

Lacouture, R. V., Johnson, J. M., Buchanan, C., & Marshall, H. G. (2006). Phytoplankton index of biotic integrity for Chesapeake Bay and its tidal tributaries. *Estuaries and Coasts*, *29*(4), 598-616.

Marshall, H.G. and T.A. Egerton. 2009. Increasing occurrence and development of potentially harmful algal blooms in Virginia tidal rivers. Conference Proceedings: Water Resources in Changing Climates. Virginia Tech Water Research Center, Richmond, VA, pp. 89-101.

Marshall, H. G., Lacouture, R. V., Buchanan, C., & Johnson, J. M. (2006). Phytoplankton assemblages associated with water quality and salinity regions in Chesapeake Bay, USA. *Estuarine, Coastal and Shelf Science*, *69*(1), 10-18.

Marshall, H.G. and R.W. Alden. 1990. A comparison of phytoplankton assemblages and environmental relationships in three estuarine rivers of the lower Chesapeake Bay. *Estuaries*, 13:287-300.

Marshall, H.G., L. Burchardt, and R. Lacouture. 2005. Phytoplankton composition within Chesapeake Bay and its tidal estuaries. *Journal of Plankton Research*, 27(11):1083-1102.

Marshall, H.G., M. Lane, and K. Nesius. 2003, Long-term phytoplankton trends and related water quality trends in the lower Chesapeake Bay, Virginia, U.S.A. Environmental Monitoring and Assessment, 81:349-360.

Marshall, H.G., M. Lane, K. Nesius, and L. Burchardt. 2009. Assessment and significance of phytoplankton species composition within Chesapeake Bay and Virginia tributaries through a long-term monitoring program. *Environmental Monitoring and Assessment*, 150:143-155.

Moore, K.A., D. B. Parrish, and B. B. Neikirk. 2013. Fulfilling data needs for assessing numeric CHL*a* criteria of the lower James River Estuary. Report to the Virginia Department of Environmental Agency. Virginia Institute of Marine Science, Gloucester Point, Virginia.

Morse, R. E., J. Shen, J. L. Blanco-Garcia, W. S. Hunley, S. Fentress, M. Wiggins, and M. R. Mulholland. 2011. Environmental and physical controls on the formation and transport of blooms of the dinoflagellate *Cochlodinium polykrikoides* Margalef in the lower Chesapeake Bay and its tributaries. *Estuaries and Coasts,* 34: 1006-1025.

Mulholland, M. R., Morse, R. E., Boneillo, G. E., Bernhardt, P. W., Filippino, K. C., Procise, L. A., Blanco-Garcia, J.L, Marshall, H.G., Egerton, T.E., Hunley, W.S., Moore, K.A., Berry, D.L. and Gobler, C. J. 2009. Understanding causes and impacts of the dinoflagellate, Cochlodinium polykrikoides, blooms in the Chesapeake Bay. *Estuaries and Coasts*, *32*(4), 734-747.

Nesius, K. K., Marshall, H. G., & Egerton, T. A. (2007). Phytoplankton Productivity in the Tidal Regions of four Chesapeake Bay (USA) Tributaries. *Virginia Journal of Science*., 58(4), 191.

Pitcher, G.C. 2012. Harmful algae- the requirements for species-specific information. *Harmful Algae.* 14, 1-4.

Pielou, E.C. 1966. Species-diversity and pattern-diversity in the study of ecological succession. *Journal of Theoretical Biology*. 10, 370-383.

Smayda, T. 1978. From phytoplankters to biomass. Pages 273-279 in A. Sournia editor. Phytoplankton manual. UNESCO, Paris, France.

Smayda, T. 1997. What is a bloom? A commentary. *Limnology and Oceanography*, 42(5), 1132-1136

Thessen, A. E., & Stoecker, D. K. (2008). Distribution, abundance and domoic acid analysis of the toxic diatom genus Pseudo-nitzschia from the Chesapeake Bay. *Estuaries and Coasts*, *31*(4), 664-672.